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POSTER ABSTRACTS

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POSTER AWARD WINNER

Functionalized Gold Nanoparticles as a Targeted X-ray Contrast Agent for Damaged Bone Tissue

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The role of microdamage in clinical bone fragility remains poorly understood due, in part, to limitations in available methods to detect and image damage, which are inherently two-dimensional, destructive, invasive and tedious. Therefore, gold nanoparticles (Au NPs) have been investigated as a targeted X-ray contrast agent due to their relative biocompatibility, ease of surface functionalization, colloidal stability and high X-ray attenuation. Au NPs were synthesized using the citrate reduction method to a mean particle diameter of 12 nm (TEM, DLS). Particle surfaces were functionalized with either L-glutamic acid (GA), 2-aminoethylphosphonic acid (PA), or alendronate (BP) (FT-IR), which exhibit a primary amine for binding gold opposite carboxylate, phosphonate, or bisphosphonate functional groups, respectively, for targeting calcium in damaged tissue. Surface functionalization efficiency was compared using mass spectroscopy. Functionalized Au NPs exhibited high colloidal stability (UV-vis) with a negative surface charge (zeta potential). The binding affinity of functionalized Au NPs for hydroxyapatite, as a synthetic bone mineral analog, was quantified by linearizing the resulting Langmuir binding isotherms. BP-Au NPs exhibited the highest binding affinity and the most rapid binding kinetics, followed by GA- and PA-Au NPs, respectively. The differences in binding efficiency were not correlated with surface functionalization efficiency, suggesting that the binding affinity of the functional group and not the number of functional groups present led to the high binding affinity of BP-Au NPs. Binding affinity to mineralized tissue was compared using cortical bone specimens that were artificially damaged and subsequently labeled with functionalized Au NPs. Qualitative (light microscopy, backscattered SEM) and quantitative (XRF) comparisons were in agreement with the relative binding affinity for hydroxyapatite and further verified the specificity of functionalized Au NPs for exposed damage within bone tissue. Additionally, non-invasive, three-dimensional imaging was investigated by synchrotron-radiation computed tomography using absorption edge subtraction about the L_{III} edge of gold. Preliminary X-ray imaging studies were performed to demonstrate the ability of BP-Au NPs to target fatigue microdamage within machined human cortical bone specimens loaded in cyclic uniaxial tension. In summary, functionalized Au NPs were demonstrated to enable targeted labeling for contrast-enhanced X-ray imaging of mineralized tissues.

POSTER AWARD WINNER

An Artificial Perivascular Niche to Explore the Molecular Regulation of Adult Stem Cells

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Stem cell niches are composed of numerous components, including soluble and insoluble factors, cues from other cells, and the extracellular matrix (ECM), which collectively serve to maintain stem cell quiescence and direct differentiation. Many adult stem cell niches reside in proximity to the vasculature in vivo, a feature common to neural stem cells, mesenchymal stem cells (MSCs) from bone marrow, hematopoietic stem cells, and many tumor stem cells. We have developed a microfluidic platform to micropattern endothelial cells (ECs) and stromal cells (either fibroblasts or bone marrow-derived MSCs) in distinct 3D culture compartments. We have previously shown that both fibroblasts and MSCs are capable of supporting capillary morphogenesis in 3D fibrin gels, and that both are capable of acting as pericytes that express α smooth muscle actin. The capillary networks formed in the presence of these two different stromal populations within our microfluidic devices also possess similar morphological characteristics. ECs suspended within fibrin gels patterned in the device adjacent to stromal cells formed a primitive vascular plexus, and matured into robust capillary networks with hollow well-defined lumens. Both MSCs and fibroblasts occupied the perivascular space and formed pericytic associations with the ECs. Biochemical assays of both EC-MSC and EC-fibroblast co-cultures revealed that the interaction between α6β1 integrin receptor and EC-deposited laminin is a feature unique to MSCs in our model system. This result is consistent with published in vivo studies suggesting this integrin is required for the perivascular association of other adult stem cells in the body. We are currently investigating whether these α6β1 integrin-mediated interactions between MSCs and their perivascular niche regulate the MSCs' multilineage potential. A better understanding of the molecular players involved in the regulation of adult stem cells achieved through this study may facilitate efforts to engineer more physiologically-relevant artificial stem cell niches capable of instructing adult stem cells.

POSTER AWARD WINNER

Generation of functional mesenchymal stem cells from human induced pluripotent stem cells cultured in xeno-free and defined conditions

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The promise that cells derived from pluripotent stem (PS) cells can be used in regenerative medicine has advanced significantly. Advancements in stem cell research have suggested that human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) can serve as a potential source of mesenchymal stem cells (MSCs) for clinical bone regeneration strategies. It is also imperative to develop standard culture conditions that include the use of defined substrates without the addition of xenogeneic factors. To overcome this obstacle, we recently reported the development of a defined culture system for hESCs and hiPSCs using xeno-free medium with a fully defined synthetic polymer coating poly[2-(methacryloyloxy)ethyl dimethyl-(3-sulfopropyl)ammonium hydroxide] (PMEDSAH). Analysis showed that hESCs and hiPSCs proliferate and maintain an undifferentiated state as evidenced by expression of pluripotent markers Oct-4 and Nanog, and exhibit a normal karyotype after multiple passages. Therefore, the goals of this study were: 1. To determine whether MSCs could be derived from hESCs and hiPSCs grown on PMEDSAH; and 2. To determine if the MSCs participate in the bone formation process when implanted into mouse calvaria defects. Both hESCs and hiPSCs were cultured on PMEDSAH and then induced to differentiate into MSCs, characterized by expression of markers (CD166+, CD105+, CD73+, CD44+, CD34- and CD45-) and their ability to differentiate *in vitro* into osteoblasts, chondrocytes and adipocytes. Derived MSCs were differentiated toward osteoprogenitors in medium containing osteogenic supplements for 7 days and then implanted into critical sized calvarial defects of SCID mice for 8 weeks. Histology demonstrated *novo* bone formation in the mouse calvaria, and furthermore, human nuclear antigen staining confirmed the human origin of the regenerated bone in the case of both cell sources, suggesting that hESCs and hiPSCs grown on PMEDSAH can differentiate into functional MSCs *in vivo* bone regeneration capacity.

Aggregation behavior and kinetics of bovine insulin

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Insulin is an amyloidogenic protein associated with the occurrence of diabetes. Pharmaceutical insulin prescribed for patients with diabetes is often composed of an analog of human insulin along with a stabilizing agent. Despite including stabilizing agents such as metacresol, pharmaceutical insulin is in danger of degrading, unfolding or becoming less effective, and must be stored in specific conditions and used within 30 days (on average) of opening the vial. We have used rheology and small-angle x-ray scattering (SAXS), as well as AFM, to study the aggregation kinetics of bovine insulin protein in solution at a variety of concentrations and environmental conditions, including heating rate, incubation temperature and strain. Additionally, it was found that the concentration of insulin in solution has an impact on the speed and strength of the network formed. When the experimental parameters are varied, the kinetics of the association is changed. The applied shear strain was found to impact the gelation of the insulin solution in that higher shear correlates to faster gelation. The successful use of rheology in conjunction with other methods to determine the rate of insulin aggregation in denaturing conditions provides us with a means to test alternative stabilization methods for pharmaceutical insulin and other protein therapeutics in suspension.

Development of a pH-Induced Aggregation Approach to Form Hydrogel Microspheres from Active Proteins

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Protein-based hydrogel microspheres have been played an important role in diverse areas of biomaterial science including drug delivery, tissue engineering, and biosensing. In each of these approaches maintaining protein bioactivity is critical for the microspheres' successful application. Previous approaches to form hydrogel microspheres have used organic phases, surfactants, shear forces, and toxic polymerization initiators which could decrease protein bioactivity. In this study we hypothesized that hydrogel microspheres could be formed via a pH-induced protein aggregation (PIPA) process that maintains protein activity. A protein's minimum solubility normally occurs at a pH around its isoelectric point (pI), which has been attributed to the lack of electrostatic repulsion. Therefore, we reasoned that varying pH around a protein's pI could be used to modulate formation of protein aggregates, which could serve as nucleation centers in suspension polymerizations. To demonstrate the feasibility of our PIPA approach, we formed a model protein-polymer conjugate by reacting mutant calmodulin CaM(T34C, T110C) with PEG-diacrylate to render ''PEG-CaM-PEG'' conjugates with polymerizable acrylate end groups. Then, we prepared PEG-CaM-PEG solutions with varying pH around CaM's isoeletric point, 4.2. PEG-CaM-PEG conjugates prepared at pH conditions close to CaM's pI (4.2) formed microspheres upon polymerization. Microspheres formed at pH 3.2, pH 4.2, and pH 5.2 had average diameters of 2.1±0.3 mm, 2.2±0.4 mm, and 2.2±0.3 mm, respectively. The incorporated CaM was able to translate its nanometer scale conformational change to hydrogel microsphere volume changes of 42 ± 30% of initial volume. Also, when PEG-CaM-PEG microspheres were internalized by human mesenchymal stem cells they regulated the nuclear localization of the chondrogenic master regulatory transcription factor SOX9. Together, these results demonstrate that the PIPA approach can be used to form hydrogel microspheres that maintain protein activity in solution and in cells.

Uniform mineralization of porous poly(lactide-*co*-glycolide) (PLGA) scaffolds and adsorption of dual binding peptides to enhance MC3T3 adhesion

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Osteoconduction, osteoinduction and cell transplantation are important biomaterial design strategies to enhance osteogenesis. Uniform cell transplantation onto scaffolds improves bone volume fraction regenerated *in vivo*. Precipitation of a bone-like mineral (BLM) coating by immersion in a simulated body fluid (SBF) enhances osteoconduction, and adsorption of adhesive peptides can further direct osteoconduction and ultimately improve osteogenesis. The aim of this study is to design biomaterial surfaces incorporating BLM and adhesive peptides to direct uniform cell loading onto PLGA scaffolds. Static mineralization of porous PLGA scaffolds in SBF and statically seeding biomolecules results in uneven distribution and poor loading efficiency. An Instron 8251 was used to filter SBF through 85:15 PLGA scaffolds with average pore size range of 200-400μm. Temperature and pH of the solution were maintained at 37^{o}C and 6.8 respectively while a cyclic loading rate and a triangular waveform with amplitude 25.4mm and a frequency of 0.0011Hz was selected. SEM, EDX and uCT were used to characterize coverage, composition and distribution of mineral. FITC:BSA was used to demonstrate concentration and loading time effects on total peptide adsorption. Uniform adsorption of FITC:BSA was achieved using a custom designed closed loop filtration system utilizing a peristaltic pump. Mineralized scaffolds were subsequently seeded with VTKHLNQISQSY and GGRGDGGGSVTKHLNQISQSY peptides that are 1) mineral binding and 2) mineral and cell binding respectively. MC3T3s were seeded on peptide seeded scaffolds and quantified by direct counting methods. Inducing a laminar SBF flow through the pores of the scaffold resulted in even distribution of mineral. Filtration seeding demonstrated a greater efficiency of adsorption compared to static methods and cell seeding was improved on uniformly mineralized and peptide adsorbed scaffolds. These results demonstrate the use of pressure gradients to improve uniform mineral deposition and improve biomolecule adsorption on 3D tissue engineering structures.

Development of "Smart" Particles for Effective Gene Silencing in Cervical Cancer

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Background & Objective: Recent advances in drug design have led to the development of several classes of nucleic acid molecules such as plasmid DNA (pDNA), antisense oligodeoxynucleotides (ASODN), short interfering RNA (siRNA), and micro RNA (miRNA), which can be used for treatment of cancer, viral infection, cardiovascular and neurodegenerative diseases. However, cellular uptake of these macromolecules occurs by endocytosis where they accumulate in the endosomal/lysosomal trafficking pathway, which leads to their degradation and loss of therapeutic activity. The goal of this research is to develop novel "smart" polymeric carriers to enhance the intracellular delivery of nucleic acids into target cells. We have designed and synthesized a new family of "smart" degradable, pH-sensitive, membrane-destabilizing, comb-like polymers that can effectively shuttle nucleic acid molecules past the endosomal membrane and into the cytoplasm of targeted cells to interact with their molecular targets.

Purpose & Methods: We evaluated the ability of this polymer to complex anti-Bcl-2 siRNA molecules and their uptake into HeLa cells using the gel retardation assay and flow cytometry, respectively. We evaluated their stability upon incubation with serum and nuclease enzyme using the PicoGreen and gel retardation assays, respectively. We investigated the ability of these complexes to knockdown Bcl-2 expression *in vitro* using the western blot and qRT-PCR.

Results: The "smart" comb-like polymer proved to complex model siRNA molecules into serum- and nuclease-stable particles. These "smart" particles proved to selectively knockdown Bcl-2 expression by 40-50% at the protein and mRNA levels.

Conclusions: We have successfully designed and synthesized new "smart" comb-like polymers that can successfully deliver siRNA into cervical cancer cells and achieve functional knockdown of targeted gene.

Design of Environmentally Responsive Nano-particles of Elastin-like Polypeptides for use in Theranostic Applications

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Elastin-like polypeptides (ELP) are responsive polymers that change properties in response to different stimuli, such as temperature, pH, ionic strength, and/or light. These protein based polymers consist of a common repeat sequence found in elastin, a protein that gives skin and other tissues elastic properties. The responsiveness of these polypeptides is based on a transition temperature where they go from being soluble to an aggregated state. The transition temperature is dependent on the sequence, length of the polypeptide and its concentration in the solution. Since ELPs are designed from the gene level and expressed in bacterial systems, their structure, and therefore transition temperature, can be precisely controlled. This makes them good candidates for applications requiring specific transition conditions, including targeted drug delivery, tissue engineering, bio-sensing, and surface engineering. When the ELP is in its trimer form it is thought to stabilize aggregating ELP molecules, so we designed and expressed elastin-like polypeptide trimer building blocks. The ELP trimers consist of a trimer-forming oligomerization domain at the C-terminus of the ELP. The polypeptides were designed from the DNA level using recombinant molecular biology techniques and the protein was expressed in *E. coli* bacterial expression system. These reversibly assemble into spherical nano-particles in which the size and stability can be controlled by changing salt concentration of the solution and have been observed as small as 20 nm in diameter. These self-assembling nano-particles also show great potential for use in the emerging field of theranostics which seeks to combine imaging and therapy. The field of theranostics essentially aims to integrate multiple techniques in the field of imaging, drug delivery and nanotechnology to result in patient specific treatment regiments. By using our multifunctional nano-particles as platforms we hope to integrate imaging, drug delivery capabilities and targeting motifs to create fully functional options for theranostic treatments.

Aqueous two-phase flow systems for precisely controlled delivery of cells and biomolecules within microchannels

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Laminar and pulsatile flow of aqueous solutions in microfluidic channels can be useful for controlled delivery of cells and molecules. However, due to dispersion effects resulting from diffusion and turbulence, flow profiles are usually not stable over the entire length of channels. This issue is addressed partially by using oil-in-water phase systems. However, there are limitations in terms of the biocompatibility of these systems for cell culture. Here we present a fully biocompatible aqueous two-phase flow system that can be used to pattern cells within simple microfluidic channel designs, as well as deliver biochemical treatment to cells according to discrete boundaries. We demonstrate that aqueous two-phase systems are capable of precisely delivering cells as laminar patterns, or as islands by way of forced droplet formation. We also demonstrate that these systems can be used to precisely control chemical delivery to preformed monolayers of cells. Treatments containing either the actin depolymerizing agent cytochalasinD or trypsin were localized more reliably in ATPS delivery systems than in conventional buffer solutions.

Improving Electrode-based Medical Devices using Conductive Polymers

Kyle Mallires, Andrew Sereno, Sarah Spanninga, Zak King, Jeff Hendricks, Sarah Burns.
Biotectix, LLC

Implanted electrode-based biomedical devices require both efficient electrical signal transduction and direct mechanical coupling between the electrode and the target cells for both recording and stimulation. Unfortunately, today's implanted electrodes made from inorganic materials cause an adverse foreign-body immune response in the form of fibrous scar encapsulation. The high-impedance scar tissue limits the device's efficacy – severely diminishing signal transduction by separation of device and targeted cells. Bioincompatibility, associated with poor in-vivo electrical properties, represents a key weakness of implantable biomedical devices currently in use and is the foremost roadblock to successful and efficient long-term performance.

Biotectix has developed a suite of biomimetic materials based on organic conducting polymer composites capable of improving the tissue response and electrical properties of current biomedical device electrodes and enabling the smaller devices of the future. These materials, based on electrochemically synthesized poly-3,4-ethylenedioxythiophene (PEDOT), have tailorable biomechanical properties, fractal surface morphology, and bio-functionality in addition to significantly improved biologically-relevant electrical properties. Biotectix materials drastically decrease the voltages required for charge delivery, thus minimizing harmful redox side reactions and improving electrical efficiency by decreased impedance. The organic nature of the materials allows incorporation of bioactive moieties and manipulation of biomechanical properties significantly reducing the foreign-body response associated with implantation and subsequent stimulation. The electrochemical pathway employed is highly adaptable to pre-existing devices and electrode geometries without design revision.

Biotectix electrode materials include BTDOT, gelDOT, and situDOT – each offering different modes of electrode-tissue interfacing. BTDOT is a compact coating for electrical and biocompatibility improvement. GelDOT is a conductive gel coating further promoting biomechanical integration in addition to electrical improvement. SituDOT is an innovative electrode-tissue interfacing pathway in which conducting polymers are grown directly into the tissue. Biotectix has commercially deployed BTDOT on numerous devices, including neural interfaces available from Ann Arbor's NeuroNexus Technologies.

Rational Design & Synthesis of "Intelligent" Star-Shaped Polymers for Enhancing the Intracellular Delivery of Nucleic Acids

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Recent advances in drug design have led to the development of several classes of nucleic acid molecules which have the potential to treat cancer, viral infection, and cardiovascular and neurodegenerative diseases. Transforming these nucleic acid molecules into therapeutic agents with defined dosing regimens and well-characterized activity requires the development of specialized carriers that can selectively and efficiently deliver them into the cytoplasm of targeted cells. To address this need, we have designed and synthesized a new family of star-shaped β-cyclodextrin (β-CD)-based polymers that can complex silencing RNA (siRNA) molecules into "intelligent" particles that can "sense" the drop in endosomal pH and respond by destabilizing the endosomal membrane and releasing their therapeutic cargo into the cytoplasm to produce the desired therapeutic effect. Specifically, we grafted copolymers of hydrophobic hexyl methacrylate (HMA) and cationic dimethyl aminoethyl methacrylate (DMAEMA) monomers from the secondary face of the β-CD core via acid-labile hydrazone linkages. We varied the molar ratio of HMA/DMAEMA monomers, the molecular weight of the grafts, and the number of quaternized DMAEMA monomers into trimethyl aminoethyl methacrylate (TMAEMA) ones to investigate the effect of polymer structure on the complexation of model siRNA molecules into "intelligent" particles, their uptake into human cervical cancer cells (HeLa), and the associated knockdown of the targeted gene.

Fibrinolysis by Endothelial Membrane Type-1 Matrix Metalloproteinase is Required for Normal Angiogenesis Mediated by Bone Marrow-Derived Mesenchymal Stem Cells

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Vascularization of synthetic biomaterials is limited due to impeded matrix remodeling by endothelial cells (ECs). To alleviate this problem, biomimetic peptides that render the biomaterial susceptible to proteolysis can be incorporated into these scaffolds. The selection of specific protease-sensitive sequences to embed is less clear, as we have found that the proteases required in angiogenesis will vary depending on the interstitial cell type providing the pro-angiogenic cues. Using a three-dimensional, fibrin-based angiogenesis model, we sought to investigate the proteolytic mechanisms by which bone marrow-derived stem cells (BMSCs) promote angiogenesis. We find that BMSC-mediated vessel formation is dependent on the proteolytic ability of membrane type 1-matrix metalloproteinase (MT1-MMP). Knockdown of this protease results in a small network of vessels with enlarged lumens. Contrastingly, vessel morphogenesis is unaffected by the knockdown of MMP-2 and MMP-9. Furthermore, we find that BMSC-mediated vessel morphogenesis *in vivo* follows mechanisms similar to what we observe *in vitro*. Subcutaneous, cellular fibrin implants in C.B-17/SCID mice form aberrant vasculature when MMPs are inhibited with a broad spectrum chemical inhibitor, and a very minimal amount of vessels when MT1-MMP proteolytic activity is interrupted in ECs. Other studies have debated the necessity of MT1-MMP in the context of vessel invasion in fibrin, but this study clearly demonstrates its requirement in BMSC-mediated angiogenesis. From this platform we are engineering a poly(ethylene glycol)-based synthetic hydrogel scaffold that will allow us to corroborate the requirement of MT1-MMP-mediated proteolysis during angiogenesis. This approach will allow us to substantiate the necessity of MT1-MMP in BMSC-mediated angiogenesis and more clearly elucidate the function of additional proteases. These new insights may impact strategies to revascularize ischemic tissues, especially those that involve co-delivery of ECs and stromal cells, and rationally guide the design of new biomaterial scaffolds susceptible to proteolysis.

Phosphorylation of Mineral-Specific Peptide Sequences Discovered via Phage Display

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Drawbacks in clinical strategies to treat bone defects due to trauma or disease have led to advances in regenerative therapies. Biomimetically precipitated bone-like mineral (BLM) coatings mimic native bone structure and can be used to impart osteoconductivity to biomaterials. Engineering these apatite surfaces by adsorption of peptides can further enhance bone cell adhesion and osteogenesis. A peptide with high and preferential binding to BLM, VTKHLNQISQSY (VTK), was discovered using phage display. The aim of this study is to evaluate the effect of phosphorylation of VTK (pVTK) on binding to mineral substrates. Scrambled versions of both VTK and pVTK (fixed total charge, different charge distribution) were also synthesized to determine if the effects of phosphorylation were sequence specific. Phosphorylation of the end serine residues of VTK caused a 10-fold increase in adsorption to BLM compared to VTK and also bound strongly to mineral secreted by pre-osteoblastic cells (MC3T3). pVTK exhibited a dose-dependent inhibition of mineral formation by MC3T3 cells while VTK did not inhibit mineral formation at any concentration. MTT assays showed that neither VTK nor pVTK affected cell proliferation and hence inhibition of mineralization was not due to peptide toxicity. Scrambling did not cause a significant change in peptide adsorption to BLM. Scrambled pVTK showed lower dose-dependent inhibition of cell-mediated mineralization. Computational studies modeling peptide binding to hydroxyapatite confirmed high binding of pVTK to apatite (binding energy: -13.6±5.6 kcal/mol) compared to VTK (-4.4±2.8 kcal/mol), and showed that peptide scrambling did not affect the binding energies. These results show that phosphorylation can be used to tailor peptide adsorption to increase cellular adhesion and subsequent osteogenesis. Further, phosphopeptide-mediated inhibition of mineralization can be used to treat pathological calcification in soft tissues such as blood vessels and heart valves.

Development of a Physiologically Relevant *In Vitro* Model of the Blood-Brain Barrier

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The endothelial cells lining the capillaries that supply the brain with oxygen and nutrients present a highly regulated transport barrier known as the blood-brain barrier (BBB). These cells are characterized by thick cell membranes, minimal endocytic vesicles, and highly organized tight junctions that restrict molecular diffusion across the paracellular space. The integrity and function of the BBB is regulated by several environmental conditions including endothelial cell-to-cell contact, communication with other neural cells such as astrocytes, pericytes, microglia, and neurons, and local concentration of secreted chemical factors. Several groups have cultured primary and immortalized brain capillary endothelial cells to develop an *in vitro* model that mimics the BBB for the purpose of screening transport properties of new drug molecules designed for treatment of CNS disorders. However, these *in vitro* models failed to mimic the restrictive properties of the BBB due to the formation of "loose" tight junctions, lower expression of specific carriers and transporters, and limited cell viability.

We report the development of a new 3D *in vitro* model of the BBB that is characterized with improved endothelial cell polarization, enhanced formation of tight junctions, and minimal dilution of secreted chemical factors. This new 3D model proved to be 10-fold more restrictive compared to conventional endothelial monolayers. Our results collectively indicate that we have successfully developed a new *in vitro* model of the BBB that mimics its physiological barrier properties *in vivo*.

Development of Enzyme-Activated Nanoconjugates for Selective Therapy of Liver Cancer

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Current non-surgical treatment techniques for therapy of liver cancer, including chemoembolization and systemic chemotherapy, are greatly limited by severe loco-regional and systemic toxicities resulting in poor therapeutic response. There is an urgent need for novel therapeutic platforms that can selectively accumulate in tumor tissue, and deliver a large dose of chemotherapeutic agents into hepatic cancer cells to achieve high therapeutic activity while minimizing or eliminating side effects. Polyamidoamine (PAMAM) dendrimers are highly branched polymers characterized by a large number of chemical surface groups suitable for coupling of chemotherapeutic agents while retaining aqueous solubility. We report the conjugation of the anticancer agent Doxorubicin (Dox) to generation 5 (G5) PAMAM dendrimers via an enzyme-activated chemical linkage designed to be selectively cleaved by the liver-specific P450 enzymes and release free Dox to the cytoplasm of hepatic cancer cells. Three different linkage compositions were designed to achieve tunable Dox release kinetics based on susceptibility of these linkers to enzymatic reduction. Rate and extent of drug release from these conjugates in the presence of human hepatic microsomal enzymes showed an increase in Dox release as a function of linkage composition. This increase in drug release kinetics due to linkage-specificity towards enzymatic reduction was supported by an *in vitro* drug release assay after incubation with human hepatic cancer cells. Finally, the cytotoxicity profiles of these conjugates towards the hepatic cancer cells was examined and confirmed the change in drug release rate lead to differential cytotoxicity profiles towards cancer cells.

Solution Microarray for No-Wash, Low-Cost, Singleplex Detection of Graft-Versus-Host Disease Biomarkers

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Acute graft-versus-host (GVHD) disease is an immunological disorder that results in a 50% mortality rate in hematopoietic cell transplant patients. Early diagnosis and prognosis is key to minimizing disease onset but there is a lack of *in vitro* diagnostic tools that reliably detect acute GVHD. Current singleplex assays to detect these biomarkers in patient plasma have high assay costs and some require numerous wash steps, limiting widespread clinical utility of non-invasively diagnosing acute GVHD. To overcome this need, we are utilizing our aqueous two-phase system (ATPS) micropatterning technology to develop and validate a no-wash, low-cost, singleplex bead-based assay for candidate GVHD biomarkers, namely interleukin-8 (IL-8). Our assay consists of 7 %(w/w) poly(ethylene glycol) and 7 %(w/w) dextran as the constituent phases.

Investigating the roles of nanotopography and substrate wettability on osteoblasts

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Physical and chemical cues intrinsic to the extracellular matrix (ECM) can vary over several orders of magnitude. ECM fibers range from nanometers in width to several millimeters in length, and their topography has been hypothesized to influence cell behavior. Recently, topography has been shown to produce a quasi-3D cellular response independent of ligand type and density. However, the role of topography on cell adhesion, and on events downstream of adhesion, remains incompletely understood. Cell adhesion may be influenced by surface chemistry or topography in part by affecting the wettability of a surface of interest, which in turn may affect the identity and quantity of proteins that adsorb. To better understand the complex interplay between substrate topography, wettability, cell adhesion, and cell function, we manufactured patterned poly(methyl methacrylate) (PMMA) substrates with nanoscale gratings using nanoimprint lithography. We then investigated the response of MC3T3-E1, a mouse preosteoblastic cell line, to nanoscale gratings of different sizes. In general, nanotopography induced cellular alignment, actin alignment, and brought about differences in vinculin association in focal adhesions relative to cells cultured on smooth surfaces. Gratings of widths between 140 and 415 nm caused reduced proliferation at early time points ($t<7$ days). Furthermore, cells in osteogenic media on nanoimprinted PMMA exhibited a more differentiated phenotype based on a greater degree of mineralization than did those cultured on unpatterned surfaces. In addition, measurements of substrate wettability suggested that more hydrophobic surfaces induced greater bone formation. Based on these results, we suggest that different sizes of topography may differentially affect protein adsorption, which in turn may alter cell adhesion and signaling downstream of adhesion. Ongoing work seeks to further investigate this possibility.

Manufacturing a gastrointestinal hollow organ using physiologically functional bioengineered circular smooth muscle constructs on a novel biodegradable chitosan scaffold

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Background: The GI tract is a complex hollow structure with multiple layers of muscularis propria sandwiching neural plexuses. We have previously bioengineered physiologically functional constructs of concentrically aligned circular smooth muscle. Objective: To engineer a biodegradable, biocompatible tubular construct for intestinal implantation. Methods: The main structural component of the scaffold was chitosan crosslinked with collagen and Heparan Sulfate to obtain the final composite chitosan. 1) 2D matrices were formed using the composite to assess biocompatibility with intestinal smooth muscle cells. 2) Composite chitosan 3D tubular scaffolds were formed by freezing and lyophilizing. 3) Three bioengineered tissue constructs were mounted around the scaffold and their physiological functionality was assessed after two weeks by measuring their force generation. Results: (1) Microscopic analysis of the cells cultured on composite chitosan indicated that cells attached to the matrix and maintained their spindle-like morphology. Cells stained positive for contractile smooth muscle markers α-smooth muscle actin and smooth muscle specific caldesmon. (3) Fabricated scaffolds had different lengths, wall thicknesses and lumen diameters. The pore size ranged from 5μm to 100μm, determined by scanning electron microscopy. (4) Force generation analysis showed that tissue constructs established baseline as compared to control and maintained physiological functionality *(i)* KCl caused a rapid contraction of up to 90μN; Acetylcholine also induced a rapid onset contraction of up to 50μN; *(ii)* Relaxation of up to 60μN was induced by Vasoactive Intestinal Peptide (VIP). Conclusion: Chitosan was non-toxic to intestinal smooth muscle cells and provided a good matrix for their survival and maintenance of their contractile phenotype. The tissue constructs maintained their response to contractile and relaxant agonists. This is the first report of bioengineering circular smooth muscle tissue constructs on biodegradable chitosan scaffolds. This study provides a basis for regenerating a functional GI construct. *Supported by NIH1RC1DK087151.*

Assessing the Permeability of Engineered Capillary Networks

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Poor blood vessel growth is a characteristic of several common pathologies, which results in reduced delivery of nutrients and oxygen to the surrounding tissue, introducing a need for tissue engineered blood vessels. Distributing endothelial cells (ECs) and supporting stromal cells in a 3-D fibrin matrix has allowed our lab to develop a simple method to grow capillaries *in vitro*. Upon implantation *in vivo*, these developing vessels will anastamose with the host vasculature to restore blood flow to the desired area. However, if the engineered vessels contain endothelial cells that are misaligned, stacked, or contain wide junctional gaps, they may fail to function as a selectively permeable membrane and behave more like the pathologic vessels that supply nutrients to tumors, with turbulent flow and increased interstitial pressure. The purpose of this study was to test the resistance to permeability of these networks, grown with different stromal cell types, as a metric of vessel functionality. Our results indicate that vessel permeability decreases over time as the vessels are allowed to mature, but the ultimate degree of permeability depends on the identity of the stromal cells in co-culture with the ECs. We arrived at this conclusion by performing a series of *in vitro* experiments in which a fluorescently tagged 70 kDa dextran tracer was used to characterize vessel permeability of fixed cultures. The fluorescent signal was quantified by pixel comparison via a customized MATLAB algorithm. During the first 5 days of *in vitro* growth, capillary permeabilities were high in all conditions, due to the immaturity of the nascent vessels. At later time points, the dextran accumulated predominantly at the vessel periphery. Quantitatively, the permeability of fibroblast-EC co-cultures dropped from initial values of 61% to 39% after 7 days, and to 7% after two weeks. By comparison, vessels formed from co-cultures of ECs and either bone marrow-derived mesenchymal stem cells (MSCs) or adipose-derived stem cells (AdSCs) achieved much tighter control of permeability more quickly. Relative to fibroblast-EC co-cultures, permeability levels decreased by 68% for MSC-EC co-cultures and 77% for AdSC-EC co-cultures after 14 days of culture. These data suggest that the rates at which engineered capillary networks mature and attain functional properties depend heavily on the identity of the stromal cells.

Development of Photopolymerizable PEG-Protein Hydrogels for 3D Cell Culture

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The cellular microenvironment relies on a complex interplay between soluble factors, external forces, and extracellular matrix. An approach towards simulating the *in vivo* environment has been to employ three-dimensional cell culture; in particular, by engineering an extracellular matrix which can support cell adhesion, migration, proliferation, and differentiation. A synthetic matrix enables control over microarchitecture and resulting physical properties but these approaches often suffer from poor cell viability when compared to naturally-derived alternatives. This drawback is often addressed by the addition of adhesive moieties or peptide sequences to increase cytocompatibility. Following this approach, we have developed a biomaterial to address the influence of hydrogel microarchitecture on the 3D organization of endothelial cells co-cultured with fibroblasts *in vitro*. Our approach incorporates structural proteins into a synthetic matrix to produce a hybrid protein-polymer conjugate. Specifically, we covalently linked poly(ethylene glycol)-diacrylamide (PEG) to modified type-I collagen using a Michael's type addition reaction that preserves the quaternary structure of the collagen macromolecule. These PEG-collagen molecules are then photopolymerized resulting in collagen macromolecules crosslinked by PEG to produce an amorphous hydrogel microarchitecture. The physical properties of this hydrogel can be further modified through addition of exogenous PEG. To assess the cytocompatibility of these hydrogels, endothelial cells and fibroblasts were separately cultured on pre-polymerized hydrogels and demonstrated both viability (>90%) and adhesion. Both cell types were then photoencapsulated and each was observed to be viable in 3D culture after three days (80%). Finally, both cell types were combined and photoencapsulated in a co-culture. The cells were observed to organize into three-dimensional structures after 12 days in hydrogels made with <2% (wt/wt) exogenous PEG. Ongoing work will optimize the hydrogel formulation for *in* vitro tissue engineering approaches with emphasis on cellular organization and proliferation. Additional experiments will assess the suitability of this hydrogel for the culture and differentiation of additional cell types, particularly, mesenchymal stem cells. Beyond these immediate applications, the synthetic approach can be extended to other structural proteins to produce hydrogel systems that leverage their native structure.

Determining the effects of cyclic strain on angiogenesis in a 3-dimensional culture system

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Two important goals of tissue engineering are to understand how the rich patterns formed in the human body arise, and to then control the formation of such patterns to generate functional tissues for regenerative therapies. Unfortunately, efforts to form larger, more complex tissues have stumbled due in part to the inability of diffusive transport to sustain the metabolic demands of thick tissues. One solution proposed to overcome diffusion limits is to grow tissues with a pre-existing vasculature. Many soluble cues have been identified as important in vascular pattern formation, but emerging evidence suggests the mechanical environment is also important. To gain insight into how mechanical forces affect angiogenesis, we have designed a PDMS-based platform capable of housing both natural and synthetic extracellular matrices and supporting capillary morphogenesis in 3D. Deforming this platform using a custom designed linear stage allows for the application of a well-defined uniaxial strain to cells contained within the matrix. Initial studies show a robust linear relationship between the strain applied to the device and the strain propagated to the micro-scale. Cultured within 2.5 mg/ml fibrin gels in the device, human umbilical vein endothelial cells (HUVECs), supported by a monolayer of human aortic smooth muscle cells (HASMCs) at the upper surface of the gel, undergo angiogenesis and form capillaries that show unbiased, radial sprouting from the bead. Under conditions of applied cyclic strain, our preliminary data shows that new capillaries form parallel to the direction of applied strain, and that this directional biasing occurs as early as day 3 of the assay. Ongoing work seeks to investigate the mechanistic basis for these observations, focusing on a set of molecular signals known to influence the balance of forces between the cells and their surroundings and to play important roles in the angiogenic process. Understanding how manipulation of the mechanical microenvironment affects angiogenesis may lead to more effective strategies for patterning vasculature *in vitro*.

Carboxyl-Ebselen-Based Layer-by-Layer Film: A Potential Antithrombotic and Antimicrobial Coating

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Developing antithrombotic and antimicrobial coatings for blood-contacting devices such as heart valves, vascular grafts and catheters is important to reduce biomedical device complications and medical costs. Recently, the release of nitric oxide (NO), an anti-platelet agent, from polymeric materials has been shown to prevent thrombus formation on various devices that are in contact with fresh flowing blood. With a NO flux equivalent or higher than $0.5\sim4\times10^{-10}$ mol cm^{-2} min^{-1}, the flux generated by healthy endothelial cells (EC), an artificial surface is expected to exhibit non-thrombogenic properties similar to the EC layer. NO generation materials are a group of catalytic materials that are capable of converting endogenous *S*-nitrosothiols (RSNOs) into NO. Although aliphatic selenium species display RSNO decomposition activity, their *in-vivo* toxicity greatly limits their potential medical applications. Aromatic selenium species are known to be less toxic than their aliphatic counterparts. Ebselen, for example, has attracted a lot of attentions due to its low toxicity, anti-inflammatory, anti-oxidation and anti-microbial properties.

In this work, a carboxyl-ebselen derivative was covalently attached to polycationic polyethylenimine (PEI). The resulting ebselen-PEI (e-PEI) species exhibits RSNO decomposition catalytic activity, and can be further incorporated into a Layer-by-Layer (LbL) coating using alginate (Alg) as the polyanion. After a salt annealing and cross-linking step, which smooths the surface and enhances the robustness of the film, the LbL films are able to catalytically generate NO from RSNOs with minimal Se leaching. The annealed films have an exponential growth and the NO flux increases with the number of bilayers deposited. Physiological levels of NO are generated by a $(\text{e-PEI/Alg})_{50}$ LbL on a polyurethane catheter after 24 h of soaking in whole blood. When using *E-coli* as a model, the LbL films also exhibit significant antimicrobial activity, which is dependent on bilayer number, reducing agent concentration and incubation time. The LbL coatings, with both antithrombotic and antimicrobial properties, could be potential candidates for solving the biocompatibility and infection risk issues for a number of biomedical devices.

Crystal Structure of Resorbable $CaSiO_3$ Ceramics Controls Osteogenic Induction of hMSCs

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The repair and replacement of damaged or diseased human bone tissue require a stable interface between the orthopedic implant and living tissue. The goal of bone tissue-engineering is to design pro-osteogenic biomaterials that activate particular genes, promote positive cellular responses, and stimulate tissue regeneration along the interface, thus stabilizing the implant-tissue interface. These goals may be achieved by using bioceramic scaffolds that dissolve partially upon implantation and release pro-osteogenic soluble factors. Discovery of pro-osteogenic bioceramics is limited by expensive and time-intensive studies that examine soluble factors or physical surface properties of the scaffolds, ad-hoc, without a conceptual framework. A theoretical basis for predicting the osteo-conductivity (bone-bonding ability) and osteo-inductivity (bone-generating ability) of partially resorbable bioceramics would significantly increase the efficiency of developing pro-osteogenic materials. Here, we examine the effects of two partially resorbable $CaSiO_3$ bioceramic polymorphs (identical chemical composition, different crystal structure) on human Mesenchymal Stem Cell (hMSC) activities. We show that the nm-scale structure of silicate oligomers (ring versus chain) in the crystal structure of the two polymorphs controls the release rates and concentrations of soluble factors (Si, Ca and P) in cell culture medium. The soluble factors, in turn, affect osteo-conductive hydroxyapatite precipitation, and, ultimately, hMSC attachment, viability, and osteogenic differentiation. We provide detailed mechanistic links between crystal structure, soluble factors, and various activities of hMSCs, by combining cell culture, biomolecular and genetic (RT-PCR) analyses with detailed solution analyses of culture media and solid phase analyses of bioceramic surfaces from the nano- to the micron-scale. We also show that soluble Si and Ca are pro-osteo-inductive only within specific concentration ranges, thus clarifying controversy in the literature on the potential osteo-inductivity of Si. We propose that crystal structure is a fundamental material property that must be considered in the intelligent design of partially resorbable, pro-osteogenic bioceramic scaffolds for bone tissue-engineering.

Wrapping and Dispersion of Multiwalled Carbon Nanotubes Improves Electrical Conductivity of Protein-Nanotube Composite Biomaterials

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Composites of extracellular matrix proteins reinforced with carbon nanotubes (CNT) have the potential to be used as conductive biopolymers in a variety of biosensing, prosthetics, and tissue replacement applications. A key hurdle in creating such biomaterials is the difficulty in dispersing highly hydrophobic CNT in aqueous solutions. In this study, the effect of functionalization and polymer wrapping on the dispersion of multi-walled carbon nanotubes (MWNT) in aqueous solution was examined. Carboxylated MWNT were wrapped in either Pluronic® F127or in gelatin. Raman spectroscopy and X-ray photoelectron spectroscopy showed that functionalization disrupted the carbon lattice and added carboxyl groups. MWNT wrapped in polymers showed a further increase in adsorbed oxygen species, and those wrapped in gelatin also exhibited surface-adsorbed nitrogen. Settling time experiments showed that Pluronic® and gelatin wrapping markedly increased the stability of MWNT suspensions in water. Wrapping also increased the zeta potential of MWNT, with Pluronic®-wrapped nanotubes showing the greatest effect. Treated MWNT were also used to make 3D collagen-fibrin-MWNT composite materials and the effect of nanotube content on electrical impedance and fibroblast cell metabolic activity were assessed. Carboxylated MWNT resulted in a decrease in construct impedance by an order of magnitude, and wrapping with Pluronic® resulted in a further order of magnitude decrease. Functionalization and wrapping also were associated with maintenance of fibroblast function within protein-MWNT materials. These data show that increased dispersion of MWNT in protein-MWNT composites leads to higher conductivity and improved cytocompatibility. Understanding how CNT interact with biological systems is important in enabling the development of new biomedical technologies.